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Transdermal iontophoresis of flufenamic acid loaded PLGA nanoparticles

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ABSTRACT

The objective of this study was to test *in vitro* a drug delivery system that combines nanoencapsulation and iontophoresis for the transdermal delivery of lipophilic model drug using poly(lactic-co-glycolic acid) (PLGA) as the carrier polymer. Negatively charged fluorescent nanoparticles loaded with negatively charged flufenamic acid were prepared. The colloidal properties of the particles were stable under iontophoretic current (constant, pulsed and alternating) profiles and in contact with skin barrier. The release of the drug from the particles was not affected by iontophoresis and remained always limited (≈50%), leading to significantly lower transdermal fluxes across human epidermis and full thickness porcine skin compared to respective free drug formulation. From nanoparticles, pulsed current profile resulted in comparable or higher fluxes compared to constant current profile although fluorescence imaging was not able to confirm deeper distribution of nanoparticles in skin. Based on our results, there is no clear advantage with respect to drug permeation from nanoencapsulating flufenamic acid into PLGA nanoparticles compared to free drug formulation, either in passive or iontophoretic delivery regimens. However, pulsed current iontophoresis could be an effective alternative instead of traditional constant current iontophoresis to enhance transdermal permeation of drugs from nanoencapsulated formulations.

1. Introduction

For decades there has been an interest in using the skin as a port of entry into the body for the systemic delivery of pharmacologically active agents (Prausnitz and Langer, 2008). Transdermal delivery route can overcome problems related to poor gastrointestinal absorption, hepatic first-pass metabolism, and variable bioavailability. In addition, patient compliance can be improved by reducing the frequency of the dosing due to the continuous input of the drug. Although transdermal route is an attractive alternative to oral and parenteral administration, the clinical use of it has remained limited due to the formidable barrier properties of the outermost layer of skin, the *stratum corneum*. In order to overcome such resistance, a number of physical and chemical enhancement techniques have been developed (Rizwan et al., 2009). One such approach, iontophoresis, uses mild external electric current to facilitate the transport of ionized and neutral molecules across the membranes (Kalia et al., 2004).

The amount of drug delivered by transdermal iontophoresis can be directly controlled by the variables of the electric input of the iontophoretic device: current density, the application time, and the current type (Hirvonen, 2005). Typically the continuous direct current (DC) iontophoresis is considered to be the most efficient in transdermal delivery as the amount of drug molecules delivered is directly proportional to the total amount of current passed across the skin. However, long-term treatment with DC may lead to skin polarization that operates against the applied electrical field and may greatly decrease the magnitude of effective current across the skin and the efficiency of transdermal delivery of drugs by iontophoresis (Lawler et al., 1960). To avoid this, the current could be delivered in a periodic manner (pulsed current; PC) that permits the skin to depolarize during the "off periods" of the pulse, leading to a state that no residual charges remain in the skin by the start of the next pulse (Zakzewski et al., 1992). Another limiting factor on the efficacy of both DC and PC iontophoresis is the accumulation of small ions (H^+ and OH^-) on the electrodes that may cause electrochemical burns at long application times or when inappropriate electrode design is used (Howard et al., 1995). The periodic reversal of current polarity during the iontophoresis (alternating current; AC) avoids electrochemical burns by maintaining the same electrochemical environment of the solution surrounding the electrodes. AC has also demonstrated reduced flux drift and reduced skin to skin variability compared to conventional DC iontophoresis (Zhu et al., 2002).

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Alternatively, the potential of drug-loaded nanosized carrier systems, such as liposomes, micelles, nanoparticles, nanoemulsions and dendrimers has obtained increasing attention in transdermal delivery (Cevc and Vierl, 2010). Possible advantages from the use of such carriers include enhancement of flux, customized drug depot location, controlled release of the drug, protection of unstable therapeutics from degradation, and even selective permeabilization of *stratum corneum*. Among others, one of the most widely used polymers in nanoparticulate formulation is poly(lactic-*co*-glycolic acid) (PLGA), which is known for its excellent biocompatibility and biodegradability (Danhier et al., 2012). There are many reports on the separate use of iontophoresis and PLGA nanocarriers in transdermal delivery, however, only few studies have been published where both of them have been used in combination for the delivery of drugs (or cosmetics) across the skin (Ito et al., 2013; Tomoda et al., 2011, 2012a; Tomoda et al., 2012b). When combining PLGA nanoencapsulation with iontophoresis in transdermal delivery more predictable and controlled drug transport could be achieved, resulting in fluxes less dependent on skin variables. The drug loaded nanoparticles could create a drug reservoir on the surface of the skin/in hair follicles; from this reservoir, the drug would be slowly released to the surface of the skin/into deeper skin layers; and once released, the iontophoretic current may carry the drug across the skin into systemic circulation.

The model drug used in our study was flufenamic acid (FFA). It is a non-steroidal anti-inflammatory drug (NSAID) of the anthranilic group with analgesic, anti-inflammatory and antipyretic properties, and is used in musculoskeletal and joint disorders, as well as periarticular and soft tissue disorders (Guinamard et al., 2013). Due to the large inter-subject variability in absorption, oral administration of FFA has been discontinued and the drug is nowadays exclusively delivered topically (Lentjes and van Ginneken, 1987). Also, transdermal application is favored because of gastrointestinal perturbations and renal damage that are typical to NSAIDs. The physicochemical parameters (MW = 281.23, pKa = 3.9, logP = 5.62) – high lipophilicity and ability to dissociate under dermal conditions – makes FFA a good drug candidate to be permeated/transported across the skin (Abignente et al., 1982; Moffat, 1986). In a study by Luengo et al., where FFA was topically delivered from 328 nm PLGA particles, the nanoencapsulation caused delayed permeation of FFA and only with incubation times >12 h, the drug showed enhanced transport from nanoparticles compared to free drug (Luengo et al., 2006). Combining iontophoretic application with FFA loaded particles could enhance FFA delivery synergistically and decrease the long lag-time caused by the nanoencapsulation.

The objective of this study was to test a drug delivery system that combines nanoencapsulation and iontophoresis for the controlled transdermal delivery of lipophilic model drug. In more detail, we aimed: (1) to develop FFA-loaded PLGA nanoparticles suitable for transdermal iontophoretic administration, in regards to drug loading, colloidal properties, stability and release kinetics, (2) to study the transdermal transport of free and nanoencapsulated FFA under passive and iontophoretic conditions in two skin barrier models of "golden standard" – human epidermis and full-thickness porcine skin, (3) to determine the effect of iontophoretic current type on the permeation of FFA from PLGA nanoparticles, and (4) to visualize the distribution of applied nanoparticles in skin after iontophoretic treatment using confocal laser scanning microscopy.

2. Materials and methods

2.1. Chemicals

Poly(lactic-co-glycolic acid) 50:50 (PLGA; Resomer® RG 503H) with a molecular weight (MW) of 24,000–38,000 Da, polyvinyl alcohol (PVA; Mowiol® 4–88), flufenamic acid, 5-fluoresceinamine (FA) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (DMAP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were at least of analytical grade. Deionized water (≥18.2 MΩ/cm of resistance; Millipore, Molsheim, France) was used to prepare all the solutions.

2.2. Fluorescent labeling of PLGA

5-fluoresceineamine-bound PLGA (FA-PLGA) was prepared based upon the method described by Horisawa et al. (Horisawa et al., 2002) and Weiss et al. (Weiss et al., 2006). Briefly, 3.07 g PLGA, 0.0583 g FA and 0.0408 g of DMAP were dissolved in 30 ml of acetonitrile and incubated at room temperature for 24 h under gentle stirring and light protection. The resulting FA-PLGA was precipitated by the addition of purified water and separated by centrifugation. The polymer was rinsed from excessive reagents (repeated dissolution in acetone and precipitation with ethanol) and then lyophilized with Alpha 2–4 LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

2.3. Preparation of nanoparticles

A preformulation study was carried out to find the suitable preparation conditions and composition for the flufenamic acid loaded FA-PLGA nanoparticles intended for our transdermal iontophoretic experiments. Different nanoparticle preparation methods (nanoprecipitation, emulsion diffusion/evaporation), surfactants, loaded drug amount and homogenization conditions were tested to find the best composition. Finally, our FA-PLGA nanoparticles loaded with FFA were prepared by the emulsion diffusion/evaporation technique. In brief, 6 mg of FFA and 50 mg FA-PLGA were dissolved in 2.5 ml ethyl acetate. This organic solution was added dropwise into 2.5 ml of an aqueous solution of 2% PVA. The mixture was then kept for 1 h under magnetic stirring at 600 rpm at ambient temperature. The resulting o/w emulsion was homogenized with Branson ultrasonifier S250D (Branson Ultrasonics Co., CT, USA) at 10% amplitude for 30 s. 20 ml of water was added and the emulsion was kept under stirring overnight to evaporate the organic solvent. For reference, standard drug-free FA-PLGA nanoparticles were prepared in the same way. The dispersions were purified using Vivaspin 20 ml ultrafiltration spin column (Sartorius, Goettingen, Germany) using centrifugation at 4000 rpm for min and were further washed three times with deionized water. 1 ml of final formulation used in all permeation experiments consisted of 17.86 mg FA-PLGA nanoparticles (containing 1 mg of FFA) suspended in 25 mM Hepes buffered saline pH = 7.4.

2.4. Characterization of the nanoparticles

The morphology of the nanoparticles was evaluated by FEI Quanta™ FEG scanning electron microscope (SEM; FEI Company, Hillsboro, OR, USA). The samples were fixed onto a two-sided carbon tape with silicone adhesive and sputtered with platinum for 25 s with an Agar sputter device (Agar Scientific Ltd., Essex, UK) prior to imagining.

The colloidal characteristics of FFA loaded FA-PLGA nanoparticles were determined by Malvern Zetasizer Nano (Malvern Instruments, Malvern, UK). To assess the loading of FFA into nanoparticles, a fixed amount of purified freeze-dried nanoparticles was dissolved in acetone (polymer and drug both soluble) and then methanol was added (only drug soluble) to precipitate the FA-PLGA. The nanoparticle dispersion was centrifuged to separate the supernatant in which the encapsulated amount of FFA was determined by high performance liquid chromatography (HPLC).

2.5. Stability assessment of nanoparticles

The stability of nanoparticles was evaluated at 32 °C as a change in hydrodynamic diameter and polydispersity index (PDI). Nanoparticle stability was tracked 1) for 24 h in 25 mM HEPES buffered saline